

In the specification:

Please amend the specification as shown:

Please delete the paragraph on page 2, lines 10-17 and replace it with the following amended paragraph:

Bovine NESP55 is proposed to be a precursor for the tetrapeptide Leu-Ser-Ala-Leu (LSAL) (SEQ ID NO: 3), which has been identified as an endogenous antagonist of the serotonergic 5-HT_{1B} receptor subtype. The serotonergic system is thought to play a role in mental disorders, particularly depression. A second amino acid sequence, GAIPRRH (SEQ ID NO: 4), present at the C-terminus of bovine NESP55 is the same as that of a peptide identified in the secretory content of chromaffin granules (Sigafoos *et al* (1993) *J Anat* **183**, 253-264). A function was not assigned to this peptide, and there has been no suggestion that either peptide is involved in obesity.

Please delete the paragraph bridging pages 2-3 (page 2, line 23 to page 3, line 16) and replace it with the following amended paragraph:

A first aspect of the invention provides a polypeptide (which, for convenience, we generally term "processed polypeptide") derivable from human NESP55 wherein the said polypeptide is derivable, or predicted from the amino acid sequence of human NESP55 to be derivable, by endoproteolytic cleavage of a polypeptide having the amino acid sequence

IRLEVPKRMDRRSRAQQWRRARHNYNDLCPPIGRRAATALLWLSCSIALLRAL
ATSNARAQQRAAAQRRSFLNAHHRSGAQVFPESPESEDHEHEEADLELSLP
ECLEYEEFFDYETESETESISETDFETEPETAPTTEPETEPEDDRGPVVPK
HSTFGQSLTQRLHALKLRS PDASPSRAPSTQEPQSPREGEELKPEDKDPARD
PEESKEPKEEKQRRRCKPKKPTRRDASPEPSKKGPIPIRRH

(SEQ ID NO: 2)

or

MDRRSRAQQWRRRARHNYNDLCPPIGRRAATALLWLSCSIALLRALATSNARAQ
QRAAAQQRRSFLNAHHRSGAQVFPESPESESDHEHEEADLELSLPECLEYEEE
FDYETESETESEIESETDFETEPETAPTTEPETEPEDDRGPVVPKHSTFGQSL
TQRLHALKLRS PDASPSRAP PSTQEPQSPREGEELKPEDKDPARDPEESKEPK
EEKQRRRCKPKKPTRRDASPESSKKGPIPIRRH

(residues 9-253 of SEQ ID NO: 2)

or of a variant thereof, wherein the polypeptide variant has an amino acid sequence which has at least 90% identity with the amino acid sequence given above.

Please delete the paragraph on page 5, lines 2-12 and replace it with the following amended paragraph:

It is particularly preferred, although not essential, that the variant (or fragment, derivative or fusion or the fusion of the variant, fragment or derivative, as discussed below) of the said polypeptide comprises the amino acid sequence LHAL (SEQ ID NO: 5) and/or the amino acid sequence GPIPIRRH (SEQ ID NO: 6). It is more preferred that the variant (or fragment, derivative or fusion, or fusion of the variant, fragment or derivative) has at least one sequence of two or more consecutive basic amino acid residues at a position equivalent to two or more consecutive basic amino acid residues present in the amino acid sequence of full length human NESP55. Such two or more consecutive basic amino acid residues are present at positions 11-12, 19-20, 34-35, 69-70, 225-227, 231-232, 235-236, 245-246 and 252-253 of full length human NESP55 as shown in Figure 1 and above.

Please delete the paragraph on page 6, lines 31-33 and replace it with the following amended paragraph:

Polypeptides which are considered to be examples of said processed polypeptides include a polypeptide consisting of the amino acid sequence LHAL (SEQ ID NO: 5) and a polypeptide consisting of the amino acid sequence GPIPIRRH (SEQ ID NO: 6).

Please delete the paragraph bridging pages 6-7 (page 6, line 34 to page 7, line 7) and replace it with the following amended paragraph:

A further preferred processed polypeptide may have the N-terminal sequence SFLN (SEQ ID NO: 7), corresponding to amino acids 71 to 74 of the amino acid sequence of human NESP55, as shown in Figure 1, or amino acids 64 to 67 of the amino acid sequence of human NESP55 shown in Hayward *et al* (1998). The said polypeptide may not comprise the amino acid sequence, for example the C-terminal amino acid sequence, GPIPIRRH (SEQ ID NO: 6). The polypeptide may have the C-terminal sequence PSKK (SEQ ID NO: 8), corresponding to amino acids 243 to 246 of the amino acid sequence of human NESP55, as shown in Figure 1, or amino acids 234 to 237 of the amino acid sequence of human NESP55 shown in Hayward *et al* (1998).

Please delete the paragraphs on page 7, lines 8-26 and replace them with the following amended paragraphs:

A further preferred processed polypeptide may have the C-terminal sequence PSKK (SEQ ID NO: 8), corresponding to amino acids 243 to 246 of the amino acid sequence of human NESP55, as shown in Figure 1, or amino acids 234 to 237 of the amino acid sequence of human NESP55 shown in Hayward *et al* (1998). The polypeptide may have the N-terminal sequence of human NESP55. This may be the N-terminal sequence of human NESP55 as translated from the most 5N in frame methionine codon (ie MDRR) (SEQ ID NO: 9) or the N-terminal sequence of human NESP55 following removal of the signal sequence (ie ATAL) (SEQ ID NO: 10), which consist of the first 36 amino acids of the sequence of human NESP55 shown in Figure 1 (or above) or the first 28 amino acids of the sequence of human NESP55 shown in Hayward *et al* (1998).

A said processed polypeptide, for example GPIPIRRH (SEQ ID NO: 6), may be useful, for example as a neuropeptide or as a ligand for a neuropeptide receptor or as a means for identifying a neuropeptide receptor. A further said processed polypeptide LHAL (SEQ ID NO: 5) may be useful, for example as a 5-HT_{1B} receptor antagonist or for identifying a 5-

HT_{1B} receptor antagonist or agonist. A said processed polypeptide or human NESP55 may be useful in medicine, for example in the treatment or prophylaxis of obesity, or in the identification or preparation of a compound that may be useful in medicine, for example in the treatment or prophylaxis of obesity. These aspects of the invention are discussed more fully below.

Please delete the paragraph bridging pages 8-9 (page 8, line 35 to page 9, line 4) and replace it with the following amended paragraph:

It will be appreciated that the processed peptides of the invention may be comprised within a further sequence such that the flanking sequences may or may not be the flanking sequences in human NESP55. For example, a further aspect of the invention provides a polypeptide containing the sequence LHAL (SEQ ID NO: 5) whether or not the flanking sequences are those flanking LHAL (SEQ ID NO: 5) in native human NESP55. A still further aspect of the invention provides a polypeptide containing the sequence GPIPIRRH (SEQ ID NO: 6) whether or not the flanking sequences are those flanking GPIPIRRH (SEQ ID NO: 6) in native human NESP55.

Please delete the paragraph on page 9, lines 5 to 22 and replace it with the following amended paragraph:

The polypeptides of these aspects of the invention typically consist of the amino acid sequence X_nLHALZ_m (SEQ ID NO: 11) or X_nGPIPIRRHZ_m (SEQ ID NO: 12) wherein X_n represents the amino acid sequence of the consecutive n amino acids immediately N terminal to the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) and wherein Z_m represents the amino acid sequence of the consecutive m amino acids immediately C terminal to the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6), wherein n and m may independently be any number between 0 and 1, 5, 10, 15, 20, 25 and 30 amino acids, preferably between 0 and 20, still more preferably between 0 and 10 amino acids. It is preferred that the amino acid

sequences X_n and Z_m are those found immediately N and C terminal, respectively, to the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) in native human NESP55. It is preferred that the amino acids are L-amino acids, in particular it is preferred that the LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) motifs consist of L-amino acid residues. It is preferred that the amino acid residues immediately flanking (such as those within 10 to 20 residues) of the motif are L-amino acids residues, but that they may be D-amino acid residues. It will be appreciated that similar peptides are included in the invention in which the core motif (such as the LHAL (SEQ ID NO: 5) and the GPIPIRRH (SEQ ID NO: 6), as described above), is another peptide which is derivable by processing of human NESP55 as described above.

Please delete the paragraph on page 9, line 23 to page 10, line 5 and replace it with the following amended paragraph:

A further aspect of the invention provides a polypeptide variant, fragment, derivative or fusion of a polypeptide having the amino acid sequence

IRLEVPKRMDRRSRAQQWRRARHNYNDLCPPIGRRAATALLWLSCSIALLRAL
 ATSNARAQQRAAAQQRSSFLNAHHRSGAQVFPESPESESDHEHEEADLELSLP
 ECLEYEEEFDYETESETESEIESETDFETEPETAPTTEPETEPEDDRGPVVPK
 HSTFGQSLTQRLHALKLRSPDASPSRAPSTQEPQSPREGEELKPEDKDPRD
 PEESKEPKKEKQRRRCKPKKPTRRDASPESPSKKGPIPIRRH

(SEQ ID NO: 2)

or a fusion of a said variant or fragment or derivative, wherein the polypeptide variant has an amino acid sequence which has at least 90% identity with the amino acid sequence given above and wherein the said polypeptide variant, derivative, fragment or fusion does not have the amino acid sequence

MDRRSRAQQWRRARHNYNDLCPPIGRRAATALLWLSCSIALLRALATSNARAQ
 QRAAAQQRSSFLNAHHRSGAQVFPESPESESDHEHEEADLELSLPECLEYEEF
 FDYETESETESEIESETDFETEPETAPTTEPETEPEDDRGPVVPKHSTFGQSL
 TQRLHALKLRSPDASPSRAPSTQEPQSPREGEELKPEDKDPRDPEESKEPKE
 EKQRRRCKPKKPTRRDASPESPSKKGPIPIRRH

(residues 9-253 of SEQ ID NO: 2)

Please delete the paragraph on page 10, lines 13-24 and replace it with the following amended paragraph:

By "fusion of said polypeptide" we include said polypeptide fused to any other polypeptide. For example, the said polypeptide may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of said polypeptide. Examples of such fusions are well known to those skilled in the art. Similarly, the said polypeptide may be fused to an oligo-histidine tag such as His6 (SEQ ID NO: 17) or to an epitope recognised by an antibody such as the well known Myc tag epitope. Fusions to any variant, fragment or derivative of said polypeptide are also included in the scope of the invention. It will be appreciated that fusions which retain desirable properties, such as binding properties, an ability to be cleaved by suitable proteases, and other biological functions, of hNESP55 are particularly preferred. It is also particularly preferred if the fusions are one which are suitable for use in the screening assays described later.

Please delete the paragraph bridging page 10-11 (page 10, line 31 to page 11, line 5) and replace it with the following amended paragraph:

A particular embodiment of the invention provides a substantially pure human NESP55 polypeptide which consists of a naturally occurring allelic variant of the amino acid sequence

IRLEVPKRMDRRSRAQQWRRARHNYNDLCPPIGRRAATALLWLSCSIALLRAL
 ATSNARAQQRAAAQRRSFLNAHHRSGAQVFPESPESESDHEHEEADLELSLP
 ECLEYEEFDYETESETESEIESETDFETEPETAPTTEPETEPEDDRGPVVPK
 HSTFGQSLTQRLHALKLRSPPDASPSRAPSTQEPQSPREGEELKPEDKDP~~RR~~
 PEESKEPKEEKQRRRCKPKKPTRRDASPEPSKKGPIPIRRH

(SEQ ID NO: 2)

Please delete the paragraph bridging pages 13-14 (page 13, line 19 to page 14, line 21) and replace it with the following amended paragraph:

In one preferred embodiment the polynucleotide comprises the nucleotide sequence:

GAATTCGGCTCGAGGTGCCTAAGAGGATGGATCGGAGGTCCCGGGCTCAGCAGTGGCGCC
GAGCTCGCCATAATTACAACGACCTGTGCCCGCCCATAGGCCGCCGGGCAGCCACCGCGC
TCCTCTGGCTCTCCTGCTCCATCGCGCTCCTCCGCGCCCTTGCCACCTCCAACGCCCCGTG
CCCAGCAGCGCGCGGCTGCCCAACAGCGCCGGAGCTTCCTTAACGCCCACCACCGCTCCG
GCGCCCAGGTATTCCCTGAGTCCCCCGAATCGGAATCTGACCACGAGCACGAGGAGGCAG
ACTTTGAGCTGTCCCTCCCCGAGTGCCTAGAGTACGAGGAAGAGTTCGACTACGAGACCG
AGAGCGAGACCGAGTCCGAAATCGAGTCCGAGACCGACTTCGAGACCGAGCCTGAGACCG
CCCCACCACTGAGCCCCGAGACCGAGCCTGAAGACGATCGCGGCCCGGTGGTGCCCAAGC
ACTCCACCTTCGGCCAGTCCCTCACCCAGCGTCTGCACGCTCTCAAGTTGCGAAGCCCCG
ACGCCTCCCCAAGTCGCGCGCCGCCAGCACTCAGGAGCCCCAGAGCCCCAGGGAAGGGG
AGGAGCTCAAGCCCGAGGACAAAGATCCAAGGGACCCCGAAGAGTCGAAGGAGCCCAAGG
AGGAGAAGCAGCGGCGTCGCTGCAAGCCAAAGAAGCCACCCGCCGTGACGCGTCCCCGG
AGTCCCCTTCCAAAAGGGACCCATCCCCATCCGGCGTCACTAATGGAGGACGCCGTCCA
GATTCTCCTTGTTTTTATGGATTACAGGTGCTGGAGAATCTGGTAAAAGCACCATTGTGAA
GCAGATGAGGATCCTGCATGTTAATGGGTTTAATGGAGAGGGCGGCCGAAGAGGACCCGCA
GGCTGCAAGGAGCAACAGCGATGGTGAGAAGGCAACCAAAGTGCAGGACATCAAAAACAA
CCTGAAAGAGGCGATTGAAACCATTGTGGCCGCCATGAGCAACCTGGTGCCCCCGTGGA
GCTGGCCAACCCCGAGAACCAGTTCAGAGTGGACTACATTCTGAGTGTGATGAACGTGCC
TGACTTTGACTTCCCTCCCGAATTCTATGAGCATGCCAAGGCTCTGTGGGAGGATGAAGG
AGTGCGTGCTGCTACGAACGCTCCAACGAGTACCAGCTGATTGACTGTGCCAGTACTT
CCTGGACAAGATCGACGTGATCAAGCAGGCTGACTATGTGCCGAGCGATCAGGACCTGCT
TCGCTGCCGTGTCCTGACTTCTGGAATCTTTGAGACCAAGTTCAGGTGGACAAAGTCAA
CTTCCACATGTTTGACGTGGGTGGCCAGCGCGATGAACGCCGCAAGTGGATCCAGTGCTT
CAACGATGTGACTGCCATCATCTTCGTGGTGGCCAGCAGCAGCTACAACATGGTCATCCG
GGAGGACAACCAGACCAACCGCCTGCAGGAGGCTCTGAACCTCTTCAAGAGCATCTGGAA
CAACAGATGGCTGCGCACCATCTCTGTGATCCTGTTCCCTCAACAAGCAAGATCTGCTCGC
TGAGAAAGTCCTTGCTGGGAAATCGAAGATTGAGGACTACTTTCCAGAATTTGCTCGCTA
CACTACTCCTGAGGATGCTACTCCCGAGCCCGGAGAGGACCCACGCGTGACCCGGGCCAA
GTACTTCATTTCGAGATGAGTTTCTGAGGATCAGCACTGCCAGTGGAGATGGGCGTCACTA
CTGCTACCCTCATTTACCTGCGCTGTGGACACTGAGAACATCCGCCGTGTGTTCAACGA
CTGCCGTGACATCATTACGCGCATGCACCTTCGTCAGTACGAGCTGCTCTAAGAAGGGAA
CCCCAAATTTAATTAAAGCCTTAAGCACAATTAATTAAGAGTGAAACGTAATTGTACAA
GCAGTTAATCACCCACCATAGGGCATGATTAACAAAGCAACCTTTCCCTTCCCCCGAGTG
ATTTTGCGAAACCCCTTTTCCCTTCAGCTTGCTTAGATGTTCCAAATTTAGAAAGCTTA
AGGCGGCCTACAGAAAAAGGAAAAAGGCCACAAAAGTCCCTCTCACTTTCAGTAAAAA
TAAATAAAACAGCAGCAGCAAAACAAATAAAATGAAATAAAAGAAACAAATGAAATAAATA
TTGTGTTGTGCAGCATTAATAAAAAATCAAAATAAAATTAATGTGAGCAAAAAAAAAA
AAAAAGGGCGGCCGC

(SEQ ID NO: 1)

or a variant, fragment, fusion or derivative thereof. The nucleotide sequence encoding human NESP55 is shown in Figure 2.

Please delete the paragraph on page 14, lines 22-29 and replace it with the following amended paragraph:

A full length human NESP55 polypeptide sequence may be as follows and as given in GenBank entry accession number AJ009849 (Hayward *et al* (1998) *PNAS* **95**, 15475-15480):

MDRRSRAQQWRRARHNYNDLCPPIGRRAATALLWLSCSIALLRALATSNARAQ
QRAAAQQRRSFLNAHHRSGAQVFPESPESEDHEHEEADLELSLPECLEYEEE
FDYETESETESIETDFETEPETAPTTEPETEPEDDRGPVVPKHSTFGQSL
TQRLHALKLRSPDASPSRAPSTQEPQSPREGEELKPEDKDPRDPEESKEPKE
EKQRRRCKPKKPTRRDASPESPSKKGPIPIRRH

(residues 9-253 of SEQ ID NO: 2)

Please delete the paragraph bridging pages 20-21 (page 20, line 30 to page 21, line 2) and replace it with the following amended paragraph:

A still further aspect of the invention provides an antibody reactive towards a polypeptide of the invention or a fragment thereof, for example a processed polypeptide of the invention, for example an antibody reactive towards a polypeptide consisting of the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6). It is particularly useful if the antibodies recognise and bind to an epitope within the amino acid sequences LHAL (SEQ ID NO: 5) and GPIPIRRH (SEQ ID NO: 6).

Please delete the paragraph on page 21, lines 11-22 and replace it with the following amended paragraph:

In a preferred embodiment the antibody is raised using any suitable peptide sequence obtainable from the given amino acid sequence of human NESP55. It is preferred if polyclonal anti-peptide antibodies are made. Suitable peptides obtainable from human NESP55 include LHAL (SEQ ID NO: 5) (corresponding to residues 172 to 175 of NESP55 and GPIIRRH (SEQ ID NO: 6) (corresponding to residues 247 to 254 of human NESP55). In a preferred embodiment of the invention, an antibody of the invention is capable of preventing or disrupting the interaction between a polypeptide of the invention or a fragment thereof, for example a fragment comprising the amino acid sequence LHAL (SEQ ID NO: 5) or GPIIRRH (SEQ ID NO: 6) and an interacting polypeptide, for example an interacting polypeptide identified by the method of the invention described below. Such antibodies are believed to be useful in medicine, for example in treating obesity.

Please delete the paragraph on page 21, lines 29-34 and replace it with the following amended paragraph:

The invention also provides an antibody directed against the peptide sequence GAIPRRH (SEQ ID NO: 4) for use in medicine; the use of such an antibody in the manufacture of a medicament for treating obesity, and a method of treating obesity using such an antibody. Antibodies which recognise the peptide GAIPRRH (SEQ ID NO: 4) are described in Lovisetti-Scamihorn *et al* (1999) *Brain Res.* **829**, 99-106, but they have not been used to treat obesity.

Please delete the paragraphs on page 22, lines 20-34 and replace them with the following amended paragraphs:

Thus, it will be appreciated that the processed polypeptide, for example which comprises the amino acid sequence LHAL (SEQ ID NO: 5) may be a peptidomimetic compound, as

described above. It will be appreciated that the term "polypeptide derivable from" includes the meaning of a peptidomimetic compound corresponding to a polypeptide with an amino acid sequence derived from the amino acid sequence of the said polypeptide.

A further aspect of the invention provides human NESP55 for use in medicine. By human NESP55 is included a polypeptide of the invention and human NESP55 as described herein and in Hayward *et al* (1998) and a variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative any thereof. Preferences for the said variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative are as indicated above. Thus, an embodiment of this aspect of the invention provides a processed polypeptide of the invention for use in medicine. A further embodiment provides a polypeptide consisting of the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6), or containing the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) and flanking sequences as defined above, for use in medicine.

Please delete the paragraphs on page 23, lines 5-30 and replace them with the following amended paragraphs:

NESP55 or a variant, fragment, derivative or fusion thereof, or a fusion of a variant, fragment or derivative, and peptides containing the amino acid sequences LHAL (SEQ ID NO: 5) and GPIPIRRH (SEQ ID NO: 6) and flanking sequences as defined above are believed to be especially useful in treating obesity. While not being bound by any theory as to why this may be so, we believe that it is due to the involvement of peptides derived from NESP55 in the serotonergic system. Furthermore, as will become clear from the Examples, we have surprisingly shown that NESP55 has a significantly increased level of expression in obese people compared to people with normal weight. Thus, a further aspect of the invention provides a method of treating or preventing obesity in a patient, the method comprising administering to the patient an effective amount of NESP55 as defined above.

A further aspect of the invention provides the use of NESP55 in the manufacture of a medicament for the treatment of obesity. By "NESP55" in the context of the method of treatment and in this aspect of the invention is included human, bovine and mouse NESP55 and a variant, fragment, derivative or fusion, or a fusion of a variant, fragment or derivative of any thereof. Preferences for the said variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative are equivalent to those indicated above in relation to human NESP55 with the substitution of a reference to bovine or mouse NESP55 for a reference to human NESP55 where appropriate. By human NESP55 is included a polypeptide of the invention and human NESP55 as described in Hayward *et al* (1998). By mouse and bovine NESP55 are included the mouse and bovine NESP55 sequences reported in Hayward *et al* (1998). In relation to the treatment and prevention aspects of the invention, NESP55 also includes the processed polypeptides as described above, and the (SEQ ID NO: 5) LHAL-containing, and (SEQ ID NO: 6) GPIPIRRH-containing peptides as described above.

Please delete the paragraph bridging pages 23-24 (page 23, line 34 to page 24, line 5) and replace it with the following amended paragraph:

An embodiment of this aspect of the invention provides the use of a processed polypeptide of the invention in the manufacture of a medicament for the treatment of obesity. A further embodiment provides the use of a polypeptide consisting of the amino acid sequence LHAL (SEQ ID NO: 5) or LSAL (SEQ ID NO: 3) or GPIPIRRH (SEQ ID NO: 6) or GAIPIRRH (SEQ ID NO: 4) in the manufacture of a medicament for the treatment of obesity. Similarly, these peptides are preferred peptides in the method of treatment or prevention of obesity described above.

Please delete the paragraphs on page 24, lines 6-31 and replace them with the following amended paragraphs:

A further aspect of the invention provides a method of identifying a polypeptide (interacting polypeptide) that is capable of interacting with human NESP55, or a polypeptide of the invention, for example a fragment, as discussed above, for example a processed polypeptide of the invention or the (SEQ ID NO: 5) LHAL-containing or (SEQ ID NO: 6) GPIPIRRH-containing polypeptides of the invention, or that is capable of interacting with a polypeptide containing the sequence GAIPIRRH (SEQ ID NO: 4), the method comprising the steps of (1) exposing the said human NESP55 or polypeptide of the invention or fragment, or polypeptide containing the sequence GAIPIRRH (SEQ ID NO: 4) to a test composition that may comprise a said interacting polypeptide, (2) detecting an interaction between the said human NESP55 or polypeptide of the invention or fragment or polypeptide containing the sequence GAIPIRRH (SEQ ID NO: 4) and a said interacting polypeptide and optionally (3) identifying and/or isolating the said interacting polypeptide.

Preferably the polypeptide containing the sequence GAIPIRRH (SEQ ID NO: 4) consists of that sequence.

The interaction between the human NESP55 or polypeptide of the invention or fragment or the (SEQ ID NO: 4) GAIPIRRH-containing polypeptide and the interacting polypeptide may be measured by any method of detecting/measuring a protein/protein interaction, as discussed further below. Suitable methods include yeast two-hybrid interactions, co-purification, ELISA, co-immunoprecipitation methods and cellular response assays. Cellular response assays may be carried out in adipocytes or adipocyte cell lines, or they may be carried out in adrenal cells, cells of the CNS (including neuronal and glial cells), epithelial cells (such as gastric cells). The processed polypeptides, such as those containing the sequences LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) or GAIPIRRH (SEQ ID NO: 4) are produced primarily in the adrenal gland and from neurones.

Please delete the paragraph bridging pages 24-25 (page 24, line 32 to page 25, line 6) and replace it with the following amended paragraph:

A further method of identifying the interacting polypeptide of the invention includes expression cloning which makes use of the transfection of cDNAs from a cellular source which is believed to encode the interacting polypeptide (such as a receptor) into a suitable cell line (such as a CHO cell line or Hep2A3 cell line) such that at least some of the cell lines express the interacting polypeptide. Cell lines expressing the interacting polypeptide are selected based on the ability of a radio-labelled human NESP55 or polypeptide of the invention or (SEQ ID NO: 4) GAIPRRH-containing peptide to bind to the transfected cell line but not to the non-transfected cell line.

Please delete the paragraph on page 25, lines 12-17 and replace it with the following amended paragraph:

Preferences for the polypeptide of the invention or fragment thereof, for example a processed polypeptide of the invention are as given above. It is particularly preferred that the fragment of the polypeptide of the invention consists of the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPRRH (SEQ ID NO: 6) or is a (SEQ ID NO: 5) LHAL-containing or (SEQ ID NO: 6) GPIPRRH-containing polypeptide as described above, and that the interacting polypeptide interacts with these sequences.

Please delete the paragraphs on page 27, lines 11-29 and replace them with the following amended paragraphs:

A still further aspect of the invention provides an antagonist of the interacting polypeptide. For example, the antagonist may be an antibody which binds to the interacting polypeptide and blocks the interaction between the interacting polypeptide and human NESP55 or the polypeptide of the invention (such as the processed polypeptides or (SEQ ID NO: 5) LHAL- or (SEQ ID NO: 6) GPIPRRH-containing

polypeptides) or the (SEQ ID NO: 4) GAIPRRH-containing polypeptide.

A further aspect of the invention thus provides a method of identifying a compound capable of disrupting or preventing the interaction between human NESP55 or a polypeptide of the invention, for example a fragment, for example a processed polypeptide of the invention, or a (SEQ ID NO: 4) GAIPRRH-containing polypeptide and an interacting polypeptide as defined above wherein the human NESP55 or polypeptide of the invention (including a variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative as discussed above), or a (SEQ ID NO: 4) GAIPRRH-containing polypeptide and/or an interacting polypeptide of the invention are exposed to the said compound and the interaction between the human NESP55 or polypeptide of the invention (including a variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative) or a (SEQ ID NO: 4) GAIPRRH-containing polypeptide and an interacting polypeptide of the invention in the presence and absence of the compound is measured.

Please delete the paragraph on page 28, lines 5-12 and replace it with the following amended paragraph:

The interaction between human NESP55 or a polypeptide of the invention (including a variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative as discussed above) or a (SEQ ID NO: 4) GAIPRRH-containing polypeptide and the interacting polypeptide and its disruption or prevention may be measured by any method of detecting/measuring a protein/protein interaction. Suitable methods include yeast two-hybrid interactions, co-purification, ELISA, co-immunoprecipitation methods and bandshift assays. Further suitable methods may include Scintillation Proximity Assays, as well known to those skilled in the art.

Please delete the paragraph bridging pages 28-29 (page 28, line 19 to page 29, line 4) and replace it with the following amended paragraph:

It will be appreciated that screening assays which are capable of high throughput operation will be particularly preferred. Examples may include cell based assays and protein-protein binding assays. An SPA-based (Scintillation Proximity Assay; Amersham International) system may be used. For example, beads comprising scintillant and an interacting polypeptide (which term it will be appreciated includes a polypeptide which capable of interacting with human NESP55 or a polypeptide of the invention and is a fragment of a polypeptide, for example a naturally occurring polypeptide, that is also capable of interacting with human NESP55 or a polypeptide of the invention) may be prepared. The beads may be mixed with a sample comprising, for example, human NESP55 or the polypeptide of the invention, for example a polypeptide comprising the sequence GPIPIRRH (SEQ ID NO: 6) into which a radioactive label has been incorporated and with the test compound. Conveniently this is done in a 96-well format. The plate is then counted using a suitable scintillation counter, using known parameters for the particular radioactive label in an SPA assay. Only the radioactive label that is in proximity to the scintillant, ie only that bound to the human NESP55 or polypeptide of the invention, for example the polypeptide comprising the sequence GPIPIRRH (SEQ ID NO: 6) that is bound to the interacting polypeptide anchored on the beads, is detected. Variants of such an assay, for example in which the interacting polypeptide is immobilised on the scintillant beads *via* binding to an antibody or antibody fragment, may also be used. It will also be appreciated that the assays may be performed using (SEQ ID NO: 5) LHAL-containing polypeptides and (SEQ ID NO: 4) GAIPIRRH-containing polypeptides.

Please delete the paragraph on page 30, lines 17-21 and replace it with the following amended paragraph:

A further aspect of the invention is a kit of parts useful in carrying out a method, for example a screening method, of the invention. Such a kit may comprise human NESP55 or a polypeptide of the invention, for example a polypeptide comprising or consisting of the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) and an interacting polypeptide, for example a receptor molecule.

Please delete the paragraph bridging pages 30-31 (page 30, line 24 to page 31, line 5) and replace it with the following amended paragraph:

It will be appreciated that such a compound may be an inhibitor of the formation or stability of a complex of human NESP55 or the polypeptide of the invention used in the screen, for example a polypeptide comprising or consisting of the amino acid sequence LHAL (SEQ ID NO: 5) or GPIPIRRH (SEQ ID NO: 6) with an interacting polypeptide(s), for example a receptor, and therefore ultimately a modulator of any activity of that complex, for example any signalling activity, for example protein kinase activity. The intention of the screen may be to identify compounds that act as modulators, for example inhibitors or promoters, preferably inhibitors of the activity of the complex, even if the screen makes use of a binding assay rather than an activity (for example signalling activity) assay. It will be appreciated that the action of a compound found to bind the interacting polypeptide may be confirmed by performing an assay of, for example, protein kinase activity in the presence of the compound. It will be appreciated that a compound that interacts with an interacting polypeptide that is a receptor molecule may act as an agonist or antagonist of any signalling activity of the said receptor.

Please delete the paragraphs on page 32, lines 5-23 and replace them with the following amended paragraphs:

Without being bound by any theory concerning the human receptor for the LHAL (SEQ ID NO: 5) peptide, and without prejudice to any other aspect of the invention, we believe that the LHAL (SEQ ID NO: 5) peptide interacts with human 5HT_{1B/1D} receptors. The molecular cloning and characterization of these receptors is described in Hamblin & Metcalf (1991) *Mol Pharmacol* 40, 143-148 "Primary structure and functional characterization of a human 5-HT_{1D}-type serotonin receptor"; Levy *et al* (1992) *J. Biol. Chem.* **267**, 7553-7662 "Molecular cloning of a human serotonin receptor (SI 2) with a pharmacological profile resembling that of the 5-HT_{1D} subtype"; and Jin *et al* (1992) *J. Biol. Chem.* **267**, 5735-5738 "Characterization of the human 5-hydroxytryptamine 1B receptor"; all of which are incorporated by reference. Using conventional techniques, transfected cells expressing these receptors can be made, and antibodies directed at the receptors can be made in the same way as described above with reference to the interacting polypeptides of the invention.

Thus, a further aspect of the invention provides a method of identifying a compound capable of disrupting or preventing the interaction between the peptide LHAL (SEQ ID NO: 5) and human 5HT_{1B/1D} receptor wherein the (SEQ ID NO: 5) LHAL-containing polypeptide and/or the said receptor are exposed to the said compound and the interaction between the polypeptide and the receptor is measured in the presence and absence of the compound.

Please delete the paragraph bridging pages 32-33 (page 32, line 30 to page 33, line 2) and replace it with the following amended paragraph:

A compound identified by or identifiable by the above two methods is part of the invention as is its use in treating or preventing obesity. Similarly, the invention includes the treatment or prevention of obesity using antagonists of human 5-HT_{1B/1D} receptor,

such as antibodies reactive with the receptor, and treatment or prevention of obesity using antibodies reactive with the peptide sequence LHAL (SEQ ID NO: 5).

Please delete the paragraphs on page 38, lines 30-32 and replace them with the following amended paragraphs:

Figure 1 shows a comparison between the human NESP55 amino acid sequence (hNESP55) (SEQ ID NO: 2) and ~~rat~~ bovine NESP55 (rNESP55) (SEQ ID NO: 15), as well as the consensus sequence (SEQ ID NO: 16).

Figure 2 shows the nucleotide sequence of a cDNA encoding human NESP55 (SEQ ID NO: 1).

Please delete the paragraph bridging page 39-40 (paragraph 39, line 27 to page 40, line 4) replace it with the following amended paragraph:

An EST clone was obtained and sequenced. The predicted amino acid sequence is shown in Figure 1 compared to the bovine NESP55 sequence, and the nucleotide sequence is shown in Figure 2. The cDNA appears to be full-length and has an open reading frame of 244 amino acids. The overall homology between the human and bovine sequences is 84%, strongly suggesting that it is a functional homologue of NESP55. We have called the protein human NESP55 (hNESP55). The neuropeptide 5-HT Moduline sequence, encoded by residues 170 to 180 of the full sequence, contains one amino acid change: instead of the sequence LSAL (SEQ ID NO: 3), the sequence has LHAL (SEQ ID NO: 5); compare QRLHALKLRSP (residues 170 to 180 of hNESP55; Figure 1) (SEQ ID NO: 13) and ERLSALRLRSP (bovine NESP55) (SEQ ID NO: 14).

Please delete the paragraph on page 40, lines 5-11 and replace it with the following amended paragraph:

A second bioactive peptide has been identified within the amino acid sequence of the bovine NESP55 protein. The peptide GAIPRRH (SEQ ID NO: 4) has been found in chromaffin granules but no function has been assigned. The equivalent peptide from hNESP55 has the amino acid sequence GPIPRRH (SEQ ID NO: 6). The amino acids flanking both bioactive peptides are different. Since these are the sites of endoproteolytic cleavage necessary to produce the peptides, this may represent species variability in recognition sequences; however, conservation of charge may be sufficient.

Please delete the paragraph on page 40, lines 18-22 and replace it with the following amended paragraph:

A CHO cell is transfected with the human receptor for the peptide GPIPRRH (SEQ ID NO: 6) which is expressed on the cell surface. Radiolabelled GPIPRRH (SEQ ID NO: 6) is incubated with the transfected cell and binds to the receptor. Compounds are tested to determine whether they specifically displace the GPIPRRH (SEQ ID NO: 6) peptide from the cell surface. Compounds that do are selected for further study.

Please insert pages 1-11 of the enclosed sequence listing in the specification prior to the abstract.